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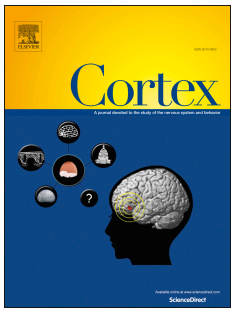

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Prefrontal activity during working memory is modulated by the interaction of variation in CB1 and COX2 coding genes and correlates with frequency of cannabis use

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Abstract

The CB1 cannabinoid receptor is targeted in the brain by endocannabinoids under physiological conditions as well as by delta9-tetrahydrocannabinol under cannabis use. Furthermore, its signaling appears to affect brain cognitive processing. Recent findings highlight a crucial role of cyclooxygenase-2 (COX-2) in the mechanism of intraneuronal CB1 signaling transduction, while others indicate that two single nucleotide polymorphisms (rs1406977 and rs20417) modulate expression of CB1 (*CNR1)* and COX-2 (*PTGS2)* coding genes, respectively. Here, our aim was to use fMRI to investigate in healthy humans whether these SNPs interact in modulating prefrontal activity during working memory processing and if this modulation is linked with cannabis use. We recruited 242 healthy subjects genotyped for *CNR1* rs1406977 and *PTGS2* rs20417 that performed the N-back working memory task during fMRI and were interviewed using the Cannabis Experience Questionnaire (CEQ). We found that the interaction between CNR1 rs1406977 and PTGS2 rs20417 is associated with dorsolateral prefrontal cortex (DLPFC) activity such that specific genotype configurations (*CNR1* C carriers/*PTGS2* C carriers and *CNR1* TT/*PTGS2 GG)* predict lower cortical response versus others in spite of similar behavioral accuracy. Furthermore, DLPFC activity in the cluster associated with the *CNR1* by *PTGS2* interaction was negatively correlated with behavioral efficiency and positively correlated with frequency of cannabis use in cannabis users. These results suggest that a genetically modulated balancing of signaling within the CB1-COX-2 pathway may reflect on more or less efficient patterns of prefrontal activity during working memory. Frequency of cannabis use may be a factor for further modulation of *CNR1/PTGS2*-mediated cortical processing associated with this cognitive process.

1. Introduction

A growing body of evidence is increasingly disambiguating the relationship between cannabinoid signaling and brain functions (Mouslech and Valla, 2009). In this regard, previous findings suggest the relevance of cannabinoids for the modulation of cognitive processing (Ruiz-Contreras et al., 2013, Ruiz-Contreras et al., 2014), as revealed by studies in humans indicating that prefrontal mediated working memory processing is compromised under acute effects of delta9-tetrahydrocannabinol (Δ9-THC) (Bossong et al., 2012a, Bossong et al., 2012b, D'Souza et al., 2012) — the main psychoactive component of cannabinoids. Consistently, other results reveal that cannabis use during adolescence is associated with increased risk for cognitive impairments in healthy subjects (Jacobus et al., 2009, Malone et al., 2010, van Winkel and Kuepper, 2014).

Cannabinoid signaling in the brain targets two pertussis toxin sensitive G-protein-coupled receptors, CB1and CB2 (Matsuda et al., 1990, Munro et al., 1993). However, CB1 is the main mediator of cannabinoids effects on brain physiology (Pertwee, 2008). This receptor is widely expressed on axons and terminals of both glial elements and neurons (Matsuda, 1997) throughout the whole central nervous system including the **dorsolateral** prefrontal cortex (DLPFC) (Pazos et al., 2005). Recent evidence has also shed light on molecular mechanisms of intraneuronal CB1 signaling transduction for which the cyclooxygenase-2 (COX-2) has a crucial role (Chen et al., 2013). In particular, previous results have indicated that pro-inflammatory and excitotoxic processes implicate stimulation of CB1 by the endogenous cannabinoid 2-arachidonylglycerol (2-AG), which in turn suppresses COX-2 activity (Zhang and Chen, 2008) *via* a mechanism mediated by the αi subunit of G protein (Zhang and Chen, 2008). However, an alternate mechanism is induced by exposure to Δ9-THC, whose stimulation of CB1 increases COX-2 transcription, expression, and activity via a mechanism mediated by the G protein βγ subunit (Chen et al., 2013). COX-2 in neurons and astroglial cells is key for the conversion of arachidonic acid (AA) into prostaglandins (Smith et al., 2000), that could in turn stimulate glutamate release in presynaptic nerve terminals and astroglial cells (Bezzi et al., 1998, Sanzgiri et al., 1999, Chen et al., 2002, Sang et al., 2005, Dave et al., 2010). Overall, this knowledge suggests that the relationship between CB1 and COX-2 is relevant for neuronal metabolism and signaling. Consistently, a recent work indicates that the CB1-COX-2 pathway activated by Δ9-THC affects synaptic plasticity and spatial

learning in mice (Chen et al., 2013). This also highlights a role of CB1 signals mediated by COX-2 in modulating complex phenotypes at the level of neuronal systems as well as of behavior.

Furthermore, evidence has indicated that genetic variation has functional consequences on expression of CB1 and COX-2 coding genes and predicts physiological, behavioral and clinical phenotypes. Specifically, a recent study (Colizzi et al., 2015) revealed that a single nucleotide polymorphism (SNP) rs1406977 (A/G) is associated with expression of the CB1 coding gene (*CNR1* - 6q14-q15) in post-mortem human prefrontal cortex as well as with cognitive behavior and related brain physiology. In particular, rs1406977 G carriers compared with AA individuals had lower prefrontal *CNR1* mRNA post-mortem expression in non-psychiatric individuals. Furthermore, G carriers who were cannabis users had greater ventrolateral prefrontal connectivity and lower accuracy during working memory than homozygous A subjects (Colizzi et al., 2015). Other studies have also indicated that a SNP (rs20417 – G>C) in the promoter region of the COX-2 gene (*PTGS2* – 1q31.1) is associated with its transcriptional activity. In particular, the C allele predicted lower COX-2 expression than the G allele in human fibroblasts (Papafili et al., 2002, Gomez-Lira et al., 2014).

The combination of information from the functional effects of genetic variation and brain imaging is a viable approach to probe *in vivo* the interaction between different partners within signaling pathways on system level phenotypes. With this perspective, the aim of this study was to investigate with fMRI in humans whether *CNR1* rs1406977 and *PTGS2* rs20417 interact in modulating **dorsolateral** prefrontal working memory processing. Given that (1) these SNPs have functional effects on gene expression, (2) COX-2 crucially participates in intraneuronal CB1 signaling transduction, and (3) cannabinoid signaling is relevant for cognitive abilities, we hypothesized a modulatory effect of *PTGS2* rs20417 on the relationship between *CNR1* rs1406977 and **dorsolateral** prefrontal working memory processing. Furthermore, we hypothesized a relationship between *CNR1/PTGS2* modulated **dorsolateral** prefrontal working memory processing and measures of cannabis use.

2. Materials and Methods

2.1 Subjects

We enrolled 242 healthy subjects (126 males, age mean ± standard deviation 26.72 ± 7.32 years). **This sample included 208 individuals investigated in a previous study** (Colizzi et al., 2015). All subjects were Caucasians from the region of Puglia, Italy. Exclusion criteria were presence of any psychiatric disorder as evaluated with the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders IV; any significant neurological or medical condition revealed by clinical and magnetic resonance imaging; history of head trauma with loss of consciousness; active cannabis use during the study protocol as well as the use of other stimulant drugs. The Wechsler Adult Intelligence Scale—Revised was used to evaluate the Intelligence Quotient (IQ), the Hollingshead Scale to calculate the socio-economical status **on the basis of educational attainment and occupational prestige** (Hollingshead and Redlich, 1958)**,** and the Edinburgh Inventory (Oldfield, 1971) to measure handedness. All participants were asked about their use of illicit drugs and those who reported cannabis use were interviewed using the Cannabis Experience Questionnaire (CEQ) (Di Forti et al., 2009, Connor et al., 2011), which allows to obtain detailed information about cannabis use habits such as lifetime use (yes/no) and frequency of use (score range: 1-5, with 1 corresponding to exposure to cannabis few times during life, and 5 corresponding to cannabis **consumption** many times a day). The CEQ administration resulted in 160 non-users and 82 users. The local institutional review board approved the protocol. After complete description of the study to all the subjects, written informed consent was obtained.

2.2 Genotype determination

Participants underwent venipuncture for subsequent DNA extraction from peripheral blood mononuclear cells. Approximately 200 ng DNA were used for genotyping analysis. DNA was concentrated at 50 ng/μl (diluted in 10 mM Tris/1mM EDTA) with a Nanodrop Spectrophotometer (ND-1000). We used Illumina HumanHap550K/610Quad Bead Chips (San Diego, California) to genotype our sample. Briefly, each sample was whole-genome amplified, fragmented, precipitated and resuspended in appropriate concentrations of hybridization buffer. Denatured samples were

hybridized on prepared Illumina Human550K/610-Quad Bead Chips. After hybridization, the Bead Chip oligonucleotides were extended by a single labeled base, which was detected by fluorescence imaging with an Illumina Bead Array Reader. Normalized bead intensity data obtained for each sample were loaded into the Illumina GenomeStudio (Illumina, v.2010.1) with cluster position files provided by Illumina, and fluorescence intensities were converted into SNP genotypes. We focused on *CNR1* rs1406977 and *PTGS2* rs20417. Given the low number of homozygous subjects for the *CNR1* rs1406977 C allele (N = 8) and for the *PTGS2* rs20417 G allele (N = 2), they were collapsed with heterozygous individuals in one group (*CNR1* rs1406977 C carriers and *PTGS2* rs20417 G carriers, respectively) for all analyses. On this basis, there were 65 *CNR1* TT/*PTGS2 CC,* 76 *CNR1* TT/*PTGS2* G carriers, 33 *CNR1* C carriers/*PTGS2* CC, and 68 *CNR1* C carriers/*PTGS2* G carriers (Table 1).

2.3 N-Back working memory paradigm

During fMRI, all subjects completed a block-designed paradigm of the N-Back task. Briefly, 'N-back' refers to how far back in the sequence of stimuli the subject had to recall. The stimuli consisted of numbers (1–4) shown in a random sequence and displayed at the points of a diamond-shaped box. The task required recollection of a stimulus seen one or two stimuli previously (1-Back or 2-Back respectively) while subjects continued to encode additionally incoming stimuli. There was also a non-memory-guided control condition (0-Back) that simply required subjects to identify the stimulus currently seen. Two different task runs were used, each alternating four 30-s blocks of a 0-Back condition with four 30-s blocks of a working memory condition (1- or 2-Back, respectively). Each run lasted 4 min and 8 s. Stimuli were presented via a back projection system, and behavioral responses were recorded through an optic fiber response box that allowed measurement of accuracy (percent of correct responses) and reaction time (milliseconds) for each trial. All subjects were trained on the task before the fMRI session.

2.4 Analysis of behavioral data

Analysis of behavioral data was performed using a factorial ANCOVA with *PTGS2* rs20417 and *CNR1* rs1406977 genotypes as independent variables, **lifetime frequency of cannabis use (0 for non**

users, 1-5 for users) as a covariate of no interest, and a score indexing behavioral efficiency during 1 and 2 back as the repeated measures factor. This score was computed as the ratio between N-back accuracy (percentage of correct responses) and reaction time (milliseconds)(Salthouse et al., 2003, Pergola et al., 2016). Thus, higher efficiency values indexed better behavioral performance. Fisher's test was used for post hoc analysis.

2.5 fMRI data acquisition and analysis

Blood oxygen level-dependent (BOLD) fMRI was performed on a GE Signa 3-T scanner (General Electric, Milwaukee, WI) equipped with a standard quadrature head coil. A gradient-echo planar imaging sequence (repetition time: 2,000 ms; echo time: 28 ms; 20 interleaved axial slices; thickness: 4 mm; gap: 1 mm; voxel size: 3.75×3.75×5; flip angle: 90°; field of view: 24 cm; and matrix: 64×64) was used to acquire 120 volumes for each task run. The first four scans were discarded to allow for a T1 equilibration effect.

Analysis of the fMRI data was completed using Statistical Parametric Mapping 8 (SPM8), http://www.fil.ion.ucl.ac.uk/spm). Images of each subject were pre-processed using the Realign and Unwarp tool within SPM8 to compensate for non-linear signal distortions potentially induced by head motion. Movement parameters were extracted to possibly exclude subjects with excessive head motion (> 3.5 mm in translation and 3.5 degrees in rotation). **None of the individuals included in this study were excluded on this basis.** Then, images were spatially normalized into the Montreal Neurological Institute (MNI) template (12-parameter affine model) and spatially smoothed (6 mm Gaussian filter). A boxcar model convolved with the hemodynamic response function (HRF) at each voxel was modeled. In the first-level analysis, linear contrasts were computed producing a t statistical map at each voxel for the 1- and 2-Back conditions, assuming the 0-Back condition as a baseline. Thus, all individual contrast images were entered in a group random-effects analysis. **Specifically, a factorial ANCOVA was performed, with *PTGS2* and *CNR1* genotypes as the between-subjects factors, N-Back load as the repeated measures factor, and lifetime frequency of cannabis use (0 for non users, 1-5 for users) as a covariate of no interest.** We used a statistical threshold of $p<0.05$, family-wise error corrected using as volume of interest the Brodmann's areas (BA) included in

bilateral DLPFC (BA9, BA10, and BA46), which is the prefrontal region mainly involved in working memory processing (Callicott et al., 1999, Zhang et al., 2007, Bertolino et al., 2009a, Bertolino et al., 2009b, Fazio et al., 2011, Colizzi et al., 2015). BAs were identified with the Wake Forest University PickAtlas (http://fmri.wfubmc.edu/cms/software#PickAtlas). **To exclude that the results were due to the inclusion of subjects with a history of cannabis use in the larger sample, we also performed with SPM8 a factorial ANOVA in the non users subsample (N= 160), with *PTGS2* and *CNR1* genotypes as the between-subjects factors, and N-Back load as the repeated measures factor. As a region of interest, we used the DLPFC cluster in which there was a significant genotype/genotype interaction in the analysis with the mixed sample (see results). A statistical threshold of p<0.05, small volume corrected within the region of interest, was used in this case.**

2.6 Correlation analysis

To explore the relationship between behavior and genetically modulated **DLPFC** activity, we performed Pearson's *r* correlation analysis entering the BOLD parameter estimates extracted with MarsBaR (http://marsbar.sourceforge.net/) as variables from the cluster in which a *PTGS2*x *CNR1* interaction was present (see 'Results') as well as the mean behavioral efficiency during 1 and 2 Back. The same BOLD parameter estimates were further used in a Pearson's test exploring the relationship between **DLPFC** activity and frequency of cannabis use **in cannabis users,** as measured with the CEQ.

3. Results

3.1 Behavioral data

Genotype groups were matched for age, IQ, handedness (all F< 0.7; all p > 0.05), gender (χ^2 <2.89; p> 0.05), cannabis use (χ^2 <7.4; p>0.05) as well as cannabis frequency of use (p>0.05). Factorial ANCOVA on behavioral efficiency at the N-back task indicated a main effect of *CNR1* rs1406977 **(F = 4.3; p = 0.04)**, with TT subjects having better efficiency scores than C carriers. Furthermore, there was neither an effect of *PTGS2* rs20417 **(F = 2.4; p = 0.1)** nor a *PTGS2* rs20417 by *CNR1* rs1406977 interaction **(F = 0.004; p = 0.9)**. Moreover, no significant interactions between genotypes and working memory load were found (all F < 1; all p > 0.05).

3.2 fMRI data

ANCOVA on imaging data revealed a main effect of *PTGS2* rs20417 in right BA10 (x: 32, y: 52, z: 32; K = 149; Z = 4.21) with *PTGS2* rs20417 C carriers having greater activity when compared to GG individuals. No main effect of *CNR1* rs1406977 was found. Furthermore, there was an interaction between *PTGS2* rs20417 and *CNR1* rs1406977 in BA9 (x: 52, y: 26, z: 36; K = 139 ; Z = 4.27) (Fig. 1a). Here, post hoc analysis on parameter estimates extracted from the significant cluster revealed that in the context *of PTGS2* rs20417 C carriers, C carriers for *CNR1* rs1406977 have lower BOLD responses relative to TT individuals; conversely, in the context *of PTGS2* rs20417 GG individuals, *CNR1* rs1406977 TT subjects have lower BOLD responses relative to individuals carrying the C allele (all p< 0.033; fig.1b). There were no genotype by load interactions.

The interaction between *CNR1* rs1406977 and *PTGS2* rs20417 on the BA9 cluster found with the investigation of the whole sample was also present when investigating the sample of non-users only (x: 52, y: 26, z: 36; K = 53; Z = 3.77). Here, post hoc analysis indicated that patterns of BOLD responses as a function of *PTGS2* and *CNR1* genotypes overlapped with those revelaed by the analysis on the entire sample (all p<0.049).

3.3 Correlation analysis

Pearson's test indicated a negative correlation between BOLD parameter estimates extracted from the cluster in BA9 associated with a significant *CNR1* by *PTGS2* interaction and the behavioral efficiency index in the entire sample (r = -0.23; p = 0.0001; Fig.2). Furthermore, BA9 parameter estimates were also positively correlated with frequency of cannabis use **in cannabis users** (r = 0.34; p = 0.004; Fig. 3).

4. Discussion

We found that the interaction between two functional polymorphisms predicting expression of *CNR1* and *PTGS2* genes is associated with **dorsolateral** prefrontal activity during a working memory task. This finding suggests for the first time that genetic variations affect signaling of the CB1-COX2 pathway and interact in modulating **dorsolateral** prefrontal processing during working memory in healthy humans. The data also suggest possible mechanisms by which cannabinoid signaling may modulate **dorsolateral** prefrontal working memory processing in humans.

The DLPFC is a working memory related brain region (Callicott et al., 1999, Bertolino et al., 2009a, Bertolino et al., 2009b) where both CB1 and COX-2 are highly expressed (Lichtman and Martin, 1996, Minghetti, 2004, Hoffman et al., 2007, Puighermanal et al., 2009, Fan et al., 2010, Han et al., 2012). In particular, our results indicate that modulation of DLPFC BOLD activity by *CNR1* rs1406977 genotypes is differentially shaped as a function of *PTGS2* rs20417 in the absence of a genotype/genotype interaction on behavioral data. In more detail, C carriers for *CNR1* rs1406977 had lower BOLD responses relative to *CNR1* TT individuals in the context of *PTGS2* rs20417 C carrying subjects. On the other hand, an opposite pattern was present in the context of *PTGS2* rs20417 G homozygosity. There were lower BOLD responses in *CNR1* TT subjects compared to individuals carrying the C allele.

These results may be interpreted considering previous findings indicating that physiological activation of CB1 receptors induces COX-2 suppression (Zhang and Chen, 2008) and others suggesting that *CNR1* rs1406977 and *PTGS2* rs20417 genetically modulate CB1 and COX-2 expression, respectively (Papafili et al., 2002, Chen et al., 2013, Gomez-Lira et al., 2014, Colizzi et al., 2015). In particular, it is possible that the different genotype configurations are associated with differential signaling within the CB1-COX-2 pathway so that DLPFC activity is more or less efficient. **In other words, *CNR1* Ccarriers/ *PTGS2* GG and *CNR1* TT/*PTGS2* Ccarriers, compared with *CNR1* Ccarriers/ *PTGS2* Ccarriers and *CNR1* TT/*PTGS2* GG, may have greater need to recruit more prefrontal resources to perform the working memory task with the same behavioral proficiency, i.e., they are associated with lower DLPFC efficiency (Callicott et al., 2003).** This interpretation is speculative in nature and awaits further experiments, but is consistent with **current models of prefrontal function underlying**

working memory (Callicott et al., 2003) and is supported by our correlation analysis indicating that the greater the **DLPFC** activity, the lower the behavioral performance during the N-back. Beyond this interpretation, these results support the involvement of CB1 in mechanisms of modulation of **dorsolateral** prefrontal function during working memory *via* its interaction with COX-2 signaling.

We also found that cannabis users have **DLPFC** activity during working memory that was positively correlated with the frequency of cannabis use. Consistent with this line of reasoning, this relationship may indicate that the greater the frequency of use of cannabis, the lower the **DLPFC** efficiency during working memory processing. Accordingly, previous findings suggested a link between increased cannabis use and inefficient **dorsolateral** prefrontal processing during working memory (Bossong et al., 2012a). Interestingly, we found this relationship in a brain region whose activity is modulated by the interaction between *CNR1* and *PTGS2* genetic variations. Thus, a further suggestion driven by our results is a link between working memory processing, habit of cannabis use, and genetic modulation of CB1/COX-2 signaling. This suggestion is also supported by knowledge indicating that activation of CB1 by Δ9-THC increases COX-2 expression and activity (Chen et al., 2013), which in turn stimulates pre-synaptic glutamate release *via* a mechanism mediated by PGE2 (Bezzi et al., 1998, Sanzgiri et al., 1999, Chen et al., 2002, Sang et al., 2005, Dave et al., 2010). Indeed, glutamate is crucially involved in working memory processing (Castner et al., 2004, Goldman-Rakic et al., 2004, Cohen Kadosh et al., 2015, Colizzi et al., 2016).

Our results did not indicate an interaction between *CNR1* rs1406977 and *PTGS2* rs20417 on behavioral efficiency during working memory. The association of genetic variation with imaging phenotypes in spite of a lack of behavioral effects is a common finding in the imaging genetics literature (Bertolino and Blasi, 2009). This suggests that imaging correlates may better capture subtle genetic effects as opposed to behavior. Indeed, the biological distance between genes effects and imaging phenotypes is shorter compared to those of behavioral phenotypes. This may explain differential power of detection of the consequences of genetic variations at the imaging vs. behavioral levels. Furthermore, in our study, we tested subtle genetic interactions that were even more difficult to detect without larger sample sizes when looking at behavioral correlates. Consistently, we found a behavioral association when testing the main effect of *CNR1* rs1406977.

One limitation of this study is that we did not test the potential interaction between *CNR1* rs1406977 and *PTGS2* rs20417 genotype, and cannabis use because the limited number of subjects would have excessively affected the statistical power of the analysis. On the other hand, we softened the potential effect of cannabis use on our data using frequency of cannabis use as a covariate in our statistical models. **More in general, a larger sample size would have further corroborated the generalizability of the results of the present study. Another possible limitation is the lack of a laboratory drug screening, which would have represented a more reliable measure of cannabis consumption than CEQ. Finally, our study does not address the relationship between genetic variation and cannabis abuse. However, this investigation was beyond our aims and should be the object of further research.**

5. Conclusion

In conclusion, our results suggest that genetic modulation of CB1/COX-2 signaling affects dorsolateral prefrontal physiology during working memory in humans and that habits of cannabis use are linked with this modulation. Further studies are needed to address the potential relevance of this signaling pathway for psychiatric conditions characterized by anomalous prefrontal processing during cognition.

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Figures and Table Caption

Table 1: Demographics of the study subjects.

Figure 1: (a) Rendered image of the brain illustrating the interaction between *PTGS2* rs20417 and *CNR1* rs1406977 on fMRI response during working memory in right BA9 (x: 52, y: 26, z: 36). **(b)** Parameter estimates extracted from the cluster illustrated in (a); **post hoc analysis revealed that in the context *of PTGS2* rs20417 C carriers, C carriers for *CNR1* rs1406977 have lower BOLD responses relative to TT individuals; conversely, in the context *of PTGS2* rs20417 GG individuals, *CNR1* rs1406977 TT subjects have lower BOLD responses relative to individuals carrying the C allele (all p< 0.033).**

Figure 2: Scatterplot of the relationship between parameter estimates extracted from the DLPFC cluster associated with a *PTGS2* rs20417 by *CNR1* rs1406977 interaction and behavioral efficiency during working memory **(r = -0.23; p = 0.0001).**

Figure 3: Scatterplot of the relationship between parameter estimates extracted from the cluster in DLPFC associated with a *PTGS2* rs20417 by *CNR1* rs1406977 interaction and cannabis use frequency **in cannabis users (r = 0.34; p = 0.004).**

CNR1 rs1406977	PTGS2 rs20417	N	M/F	Age	Socio-economic Status	Handedness	IQ	Cannabis User/Non User	Frequency of cannabis use in cannabis users
TT	GG	65	35/30	27,85 ± 6,75	42,34 ± 18,06	0,72 ± 0,42	108,51 ± 18,61	18/47	2,5 ± 1,32
TT	C carriers	76	39/37	25,88 ± 5,46	45,38 ± 16,26	0,77 ± 0,39	109,1 ± 11,31	35/41	2,43 ± 1,28
C carriers	GG	33	21/12	27,36 ± 7,7	38,35 ± 19,27	0,74 ± 0,36	109,62 ± 12,56	10/23	3,38 ± 1,5
C carriers	C carriers	68	32/36	27,18 ± 7,89	41,21 ± 16,12	0,82 ± 0,36	108,96 ± 12,70	19/49	2,94 ± 1,11
Total		**242**	**127/115**	**26,98 ± 6,87**	**42,45 ± 17,22**	**0,76 ± 0,39**	**108,95 ± 14,33**	**82/160**	**2,68 ± 1,29**